Protein kinases as targets for cancer treatment

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In various types of malignancies, conventional forms of therapy (surgery, radiation and chemotherapy) are often ineffective, as well as harmful. In the last few years, a convergence of scientific advances has enabled the identification of molecular targets and signaling pathways specific to cancer cells, resulting in therapies with enhanced selectivity and efficacy and reduced toxicity. Compound validation here has relied on target validation first, although some of the most successful drugs often have effects outside of their postulated mechanism. Protein kinases represent such molecular targets; considerable research effort has been devoted to the development of targeted drugs that inhibit the action of pathogenic kinases, and clinical studies performed so far, have validated the positive effects of kinase inhibitors for cancer treatment. In this review, the specificity, mechanism of action and antitumor activity of several new small-molecule inhibitors of tyrosine and serine/threonine kinases are discussed.

Cancer is a leading cause of death worldwide. From a total of 58 million deaths in 2005, cancer accounted for 7.6 million (or 13%) of all deaths. Based on recent estimated rates, diagnosed cancer cases will reach roughly 17 million in 2020 and 27 million by 2050 [1]. Overall, it is estimated that more than one in three people will develop some form of cancer during their life-time (38% of males and 35% of females).

Conventional management strategies in cancer therapy have been relying on surgery, radiation, hormones and chemotherapy. However, these treatments often have a narrow therapeutic index and occasionally the responses produced are only palliative, as well as unpredictable. The introduction of the concept of individualized cancer therapy, along with the development of drugs targeting molecules that are selectively altered in tumors, has provided hope for the development of more effective treatment strategies.

Progress in understanding the molecular processes underlying the development and progression of cancer has shown that protein and lipid kinases are frequently altered in tumor cells, resulting in the translation of constitutively activated proteins, and are amenable to therapeutic targeting.

Currently, protein kinases represent as much as 30% of all protein targets under investigation by pharmaceutical companies. Drugs already in clinical trials target all stages of signal transduction: from the receptor tyrosine kinases that initiate intracellular signaling through second-messenger generators and kinases involved in signaling cascades, to the kinases that regulate the cell cycle, which governs cellular fate [2–4]. Moreover, recent successful launches of drugs with kinase inhibition as the mode of action demonstrate the ability to deliver kinase inhibitors as drugs with the appropriate selectivity, potency and pharmacokinetic properties [5,6].

Based on the fundamental role of kinases in cancer progression, drugs against kinases represent a new and promising approach to cancer therapy, one that is already leading to beneficial clinical effects.

Protein kinases & diseases

Phosphorylation is considered to be one of the most common and reversible covalent post-translational modifications (Edmond H Fischer and Edwin G Krebs, Nobel Prize 1992) that may alter the activity, life span or cellular location of proteins [7,8]. Protein kinases can modulate key regulatory proteins involved in different cellular processes, including metabolism, transcription, cell-cycle progression, cytoskeletal rearrangement and cell movement, proliferation, apoptosis and differentiation. Protein phosphorylation also plays a critical role in intercellular communication during development, in physiological responses, in homeostasis and in the functioning of the nervous and immune systems [9]. It has been shown that abnormal phosphorylation of proteins can lead to the development of a number of disorders and major diseases, such as rheumatoid arthritis, cardiovascular diseases, immunodeficiency, endocrine disorders, neurodegenerative diseases and cancer [10].

Keywords: cancer, drug discovery, kinase, kinase inhibitor, phosphorylation

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Approximately 50 of the 100 known genes that have been directly linked to the induction of cancer (i.e., oncogenes) encode protein kinases. The remainder of the oncogenes specify proteins that either activate kinases or are phosphorylated by kinases. Therefore, it is not a surprise that protein kinases and phosphatases are becoming targets for drug development [11–13].

History of kinases as drug targets

Protein kinases represent the largest enzyme family encoded by the human genome, with 518 members [9] grouped into 20 known families on the basis of structural relatedness. All kinases share a highly conserved catalytic domain that spans 250–300 amino acids, but are notably different in how their catalysis is regulated. The ATP-binding pocket is between the two lobes of the kinase fold. This site, together with less conserved surrounding pockets, has been the focus of inhibitor design that has exploited differences in kinase structure and pliability in order to achieve selectivity. It was initially believed that protein kinases were not good drug targets because of the sequence, structural and mechanistic similarities shared by these enzymes, which would prevent the development of highly selective drugs with clinical potential.

The first example of compounds targeting protein kinases is represented by the monoclonal antibody trastuzumab (Herceptin®; F Hoffmann La Roche Ltd, Basel, Switzerland), which inhibits the human epidermal growth factor receptor 2 (HER-2 or ErbB2). Overexpression of the HER-2 protein correlates with poor breast cancer prognosis [14].

The first kinase inhibitor used in clinical trials was tested against chronic myelogenous leukemia (CML). The Novartis compound STI-571 (imatinib mesylate/Gleevec®) is a highly successful cancer drug owing to its activity as an inhibitor of the Abelson cytoplasmic tyrosine kinase (Abl), which is constitutively active in most patients with CML. Although it is an ATP-competitive inhibitor, analysis of the 3D structure of ABL in complex with Gleevec revealed that the drug extends much further into the catalytic site [15]. Interestingly, imatinib is also being used succesfully in gastrointestinal stromal tumors (GIST), owing to its ability to inhibit the c-KIT and PDGF-receptor tyrosine kinases [16,17].

Over the past 10 years there has been an immense growth in the development of kinase inhibitors for the treatment of cancer, chronic inflammation, metabolic disorders, neurodegenerative disease and other conditions. There are now more than 60 kinase-targeted drugs in clinical development, and many more in various stages of preclinical development. Protein kinases are rapidly becoming the major drug-discovery targets of the twenty-first century, with an estimated 30% of all efforts focusing on this class of targets [5].

Drug-development strategies for kinase inhibitors

Kinase inhibitors have been used as therapeutics for cancers resulting from constitutive kinase activation. In recent years, the main concept of selecting lead compounds for drug development has relied on target optimization.

The successful application of imatinib on CML patients could be considered unique among cancers, since this disease begins in a clinical stage caused by a single molecular abnormality. However, imatinib was initially isolated during screening for compounds that block PDGFR and was later found to also inhibit Abl and Kit kinases [18,19], resulting in wider testing in other types of cancer, including GIST and chronic myelomonocytic leukemia (CMML).

In addition, profiling of other kinase inhibitors, currently used as clinical drugs or still under development, revealed a great variety regarding their specificity for the 'originally' intended targeted kinase [20].

As kinases mediate many of the signaling pathways by which cancer cells promote their own proliferation and survival, the design of drugs targeting a unique kinase with precise specificity seems unfeasible. On the other hand, we now know more about many different kinases and their structural and functional similarities and differences. Therefore, pharmaceutical companies have currently focused on the development of drugs with multiple effects in order to overcome the fact that tumors have many, and often overlapping, biological paths that they can use to grow, resist death and spread. As more and more compounds reach the clinical-development stage, it is more essential to evaluate their toxicology and side effects against a broad panel of targets, instead of discarding them because of promiscuity against more than one kinase.

The question that arises with the arrival of multikinase inhibitors is whether it will be best to use combinational therapies of multiple targeted therapeutics or drugs such as these, with multiple effects, in order to completely eradicate a tumor and prevent resistance or relapse.

Tyrosine kinases

Classification & regulation of TK activity

Tyrosine kinases (TKs) are a family of enzymes that catalyze phosphorylation of selected Tyr residues in target proteins using ATP. The human genome contains approximately 90 TK and 43 TK-like genes. TK's are divided into two main classes: receptor protein TKs (PTKs) and cellular, or nonreceptor PTKs. Of the 91 PTKs identified thus far, 59 are receptor tyrosine kinases and 32 are nonreceptor TKs.

Receptor TKs are transmembrane proteins with a ligand-binding extracellular domain and a catalytic intracellular kinase domain, whereas nonreceptor TKs lack transmembrane domains and are found in the cytosol, the nucleus and the inner surface of the plasma membrane.

Activation of transmembrane PTKs is typically initiated by binding of a ligand (e.g., hormone or growth factor) to a specific site within the extracellular domain of the receptor. Upon ligand binding, these receptors undergo dimerization, resulting in autophosphorylation of Tyr residues within the cytoplasmic domain [21]. These phosphorylation events activate the kinase, thereby increasing its intrinsic PTK activity and produce new binding sites for intracellular adapter molecules that bring signal transduction molecules into close proximity. The nonreceptor TKs are maintained in an inactive state by cellular inhibitor proteins and lipids and through intramolecular autoinhibition [22,23]. They are activated by diverse intracellular signals through dissociation of inhibitors, by recruitment to transmembrane receptors and through transphosphorylation by other kinases.

Strict regulation of TK activity controls the most fundamental processes of cells, such as the cell cycle, proliferation, differentiation, motility and cell death or survival [24]. Unregulated activation of these enzymes can lead to various forms of cancer, as well as benign proliferative conditions. Indeed, more than 70% of the known oncogenes and proto-oncogenes involved in cancer code for PTKs.

Dysregulation of TKs in cancer

Since regulation of TKs occurs at multiple levels, it is not surprising that TKs are dysregulated in cancer cells in several ways. One of the mechanisms by which TK might acquire transforming functions is mutation. Small deletions, point mutations or even abnormal chromosomal translocations can render TKs active in the absence of a ligand, resulting in uncontrolled cell proliferation [25]. A second mechanism of TK activation results from the fusion of a receptor or a nonreceptor TK with a partner protein, which leads to constitutive oligomerization of the TK in the absence of a ligand, thereby promoting autophosphorylation and activation [26]. A third method of TK dysregulation is via autocrine–paracrine stimulation. This activation loop is stimulated when a receptor TK, its ligand, or both are overexpressed. Such cases have been immanent in a variety of human cancers [27]. Finally, impaired tyrosine phosphatase activity or decreased expression of TK inhibitors can also result in oncogenic activation of TKs [28].

TKs as targets in cancer therapy

Recently, TKs have emerged as clinically useful drug-target molecules for treating certain types of cancer (**Table 1**).

Monoclonal antibodies

Monoclonal antibodies provide a particularly attractive way to target TKs on malignant cells [29]. Antibodies can interrupt TK signaling through neutralization of ligand, blockade of ligand binding, and receptor internalization. The changes in antibody technology now allow us to produce humanized, human chimeric or bispecific antibody for targeted cancer therapy [30–31].

Trastuzumab (Herceptin®) has been shown to increase response rates and improve survival when added to chemotherapy for metastatic HER-2-overexpressing breast cancer [32]. Cetuximab (Erbitux®) is a chimeric antibody against EGFR, with activity in combination with chemotherapy in non-small-cell lung cancer (NSCLC), squamous-cell carcinoma of the head and neck, and colorectal cancer [33].

Panitumumab is a monoclonal antibody that binds to the EGFR and inhibits phosphorylation and activation of EGFR-associated kinases. It is manufactured by Amgen and was approved by the US FDA on September 27, 2006. It is used for the treatment of patients with metastatic colorectal carcinoma [34,35].

However, limitations of monoclonal antibodies are their size, which may limit tumor penetration, heterogeneous antigen expression, and expression of tumor antigens in normal cells.

Inhibitors of angiogenesis

Another class of receptor TKs targeted in anticancer-drug development are those that promote angiogenesis, particularly the VEGFR. The formation of new blood vessels is a normal aspect of embry-

Table 1. Tyrosine-kinase-targeting drugs.

Compound	Kinase target(s)	Cancer target(s)
Humanized monoclonal antibodies		
Trastuzumab (Herceptin®)	ERBB2	ERBB2+ breast cancer
Cetuximab (Erbitux®)	EGFR	Colorectal cancer, squamous cell carcinoma of head/neck
Panitumumab	EGFR	Colorectal cancer
Bevacizumab (Avastin®)	VEGFA	Colorectal cancer, NSCLC
Tyrosine kinase inhibitors		
Imatinib (Gleevec®)	ABL1/2, PDGFRα/β, KIT	CML, Ph+ B-ALL, CMML, CEL, GIST
Gefitinib (Iressa®)	EGFR	NSCLC
Erlotinib (Tarceva®)	EGFR	NSCLC, pancreatic cancer
Lapatinib (Tykerb®)	EGFR, ERBB2	Breast cancer
Dasatinib (Sprycel®)	ABL1/2, PDGFRα/β, KIT, Src family	CML
Nilotibib (Tasigna®)	ABL1/2, PDGFRα/β, KIT	CML
Sunitinib (Sutent®)	VEGFR1–3, KIT, PDGFRα/β, RET, CSF1R, FLT3	Renal cell carcinoma, GIST
Semaxanib (SU5416)	VEGFR2, KIT, EGFR	Colorectal and kidney cancer
Vatalanib	VEGFR	Colorectal cancer
Masitinib mesylate	KIT, PDGF, FGFR3	Pancreatic cancer, GIST and dermato-fibro-sarcomas
ABT-869	VEGFR, PDGFR	Breast cancer
Lestaurtinib (CEP-701)	FLT3	AML
XL-647	EGFR, HER2, VEGFR2, EphB4	NSCLC
Sorafenib (Nexavar®)	VEGFR2, PDGFRβ, KIT, FLT3, RAF1, BRAF	Renal cell carcinoma, melanoma

AML: Acute myelogenous leukemia; B-ALL: B-cell acute lymphoblastic leukemia; CEL: Chroniceosinophilic leukemia; CML: Chronic myeloid leukemia; CMML: Chronic myelomonocytic leukemia; CSF1R: Colony-stimulating factor 1 receptor; EGFR: EGF receptor; FLT3: FMS-related tyrosine kinase 3; GIST: Gastrointestinal stromal tumor; NSCLC: Non-small-cell lung cancer; PDGFR: PDGF receptor; Ph+: Philadelphia chromosome positive; VEGFR: VEGF receptor.

onic development, and a response to wounding. Cancers also stimulate angiogenesis, apparently in response to the high demand of rapidly multiplying cancer cells for oxygen and nourishment, which reach the cancer cells via the blood system.

Various angiogenesis inhibitors are currently being evaluated in clinical trials. Agents that target VEGFR signaling include the VEGF-specific antibody bevacizumab, the small-molecule TK inhibitor, such as SU5416 (semaxanib) and the aminophtlalazine PTK787 (vatalanib). Some of these agents have met with limited success in monotherapy against solid tumors [36] and appear to be more effective in combination with other agents.

Small-molecule inhibitors

The design of specific inhibitors of TKs is important both for fundamental research and for developing therapeutic strategies for the treatment of cancer. Two classes of PTK inhibitors have been developed. One acts by binding to the substrate-binding site of the enzyme (i.e., tyrphostins) and the other by binding to the ATP-binding site.

The ATP-binding site, although evolutionary conserved, can be selectively targeted by taking advantage of the minor difference in the kinase domain, which leads to changes in hydrogen bonding and hydrophobic interactions, resulting in differences of affinity [37]. One drawback of these inhibitors is that they exhibit greater cytotoxicity and can cause nonspecific inhibition of serine/threonine kinases [38].

Clinical studies conducted over the past decade have established some of these TK inhibitors as therapeutic drugs for certain cancer patients. These include imatinib/Gleevec (directed against BCR-ABL kinase), and erlotinib/Tarceva® and

gefitinib/Iressa® (directed against EGFR). However, despite the early successes, the majority of responding patients are developing resistance to these drugs [39,40].

More precisely, secondary (acquired) resistance to Gleevec develops more rapidly in patients who exhibit a clinical response during the acute phase of the disease. With Iressa and Tarceva treatment, typical remissions in drug-responsive NSCLC patients only last for 4–6 months, suggesting that secondary drug resistance often develops rapidly in that setting [41,42].

Some of the most common mechanisms that contribute to a resistance phenotype include:

- Structural alterations in the kinase domain, which result in the inability of the inhibitor to bind to and inhibit the catalytic activity of the kinase [43,44];
- Activation by tumor cells of different kinase(s) instead of the primary targeted one [45]
- Gene amplifications [46] that lead to higher expression levels of targeted kinases, resulting in the need for higher doses of the inhibitor in order to be efficient.

In light of this new challenge, agents active against new mutations that arise during therapy with first-generation TK inhibitors are being rapidly developed.

Tyrosine kinase inhibitors

Lapatinib/Tykerb®/Tycerb® or lapatinib ditosylate is a new dual TK inhibitor developed by GlaxoSmithKline as a treatment for solid tumors such as breast and lung cancer. It is an orally active small molecule that reversibly inhibits ErbB1 and ErbB2 TKs by binding to the intracellular phosphorylation domain of the receptors and preventing their autophospohorylation upon ligand binding.

Lapatinib was approved by the FDA on March 13, 2007 for use in patients with advanced metastatic breast cancer, in combination with the chemotherapy drug capectabine/Xeloda®, following data that has shown activity in patients whose disease has progressed after treatment with trastuzumab [47]. These and other observations so far [48] provide a rationale for further clinical trials of lapatinib, either as a single agent or in combination with trastuzumab in patients with HER-2-overexpressing breast cancer or in patients that have acquired resistance to trastuzumab.

Dasatinib (Sprycel®) is a new oral dual BCR-ABL and Src family (SRC, LCK, YES and FYN), TK inhibitor developed by Bristol-Myers-Squibb. It has been approved for use in patients with CML and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) with resistance or intolerance to prior therapy, including imatinib. Thanks to its less stringent binding affinity for the BCR-ABL kinase compared with imatinib/Gleevec [49], dasatinib has been shown to be active in patients with imatinib-resistant CML [50].

Nilotinib, also known as AMN107, is a high-affinity aminopyrimidine-based ATP-competitive inhibitor developed by Novartis Pharmaceuticals that is currently under investigation as a possible treatment for CML.

It has been shown that it is approximately 20-fold more potent than imatinib in the killing of wild-type BCR-ABL-expressing cells [51–53].

Early-phase clinical trials showed that nilotinib had activity in imatinib-resistant CML, and, like dasatanib, it is now approved for the treatment of resistant cases [54]. **Mention both nilotinib's and dastanib's nejm papers – ASK AUTHOR CAN THIS BE DELETED.**

Sunitinib malate (Sutent®) represents another novel oral kinase inhibitor of fms-like TK3 (Flt3), Kit, VEGF and PDGF receptors that inhibits angiogenesis and cell proliferation. Developed by Pfizer (previously Sugen), sunitinib is currently in clinical trials for treatment of a variety of malignancies, including GIST and metastatic renal cell carcinoma (RCC; kidney cancer). Evaluation of the antitumor activity of continuous daily dosing of sunitinib in patients with advanced RCC revealed tumor shrinkage in more than 80% of patients [55], while sunitinib treatment of patients who have failed prior bevacizumab-based therapy demonstrated a 56% of tumor shrinkage [56,57].

Masitinib mesylate (MM; AB1010) is a protein tyrosine kinase inhibitor which, *in vitro*, has greater activity and selectivity than imatinib mesylate (IM) against the wild-type c-Kit receptor and the mutated form in the juxtamembrane region (JM). MM also selectively inhibits the PDGF and FGFR3 receptors, while it also appears to be an inhibitor of cytochrome P450 (CYP)2C9, CYP2D6 and CYP3A4/5 in human liver microsomes.

A Phase I clinical trial of AB1010 in patients with advanced solid malignancies has shown an acceptable safety and tolerability profile, whereas one pathological partial response was observed in a patient intolerant to IM [58].

This drug is currently being investigated in several Phase II trials among a wide range of

tumor types, including pancreatic cancer, GIST and dermato-fibro-sarcomas. Preliminary results of a multicenter Phase II trial have already shown a high efficacy of MM in nonpretreated GIST patients [59].

ABT-869 is a novel multitargeted receptor TK inhibitor that has been shown to inhibit members of the VEGF and PDGF receptor families. In addition, it has less activity against unrelated receptor TKs, soluble TKs and serine/threonine kinases, and exhibits potent antiproliferative and apoptotic effects on tumor cells dependent on mutant, constitutively active, FLT3 and KIT kinases. Preclinical studies performed so far support the use of ABT–869, alone or in combination with paclitaxel, in treating breast [60]. Moreover, recent studies revealed that ABT-869 has potent activity against acute myelogenous leukemia (AML) cell lines [61], providing the basis for the clinical development of ABT-869 in the treatment of AML patients.

CEP-701 (lestaurtinib) represents an orally bioavailable indolocarbazole derivative that binds potently and specifically to FLT3 kinase, a member of the type III receptor TK family, which includes c-Kit, PDGFR and M-CSF receptors. CEP-701 inhibits autophosphorylation of FLT3, resulting in inhibition of FLT3 activity. This compound is currently in a Phase 2/3 clinical trial against AML [62,63].

XL647 is an orally bioavailable inhibitor of EGFR, HER2, VEGFR2 and EphB4 receptor TKs (RTKs) that is developed by Exelixis. XL647 demonstrates excellent activity in target-specific cellular functional assays, and has shown sustained inhibition of target RTKs *in vivo* following a single oral dose. XL647 has been well tolerated in recent Phase I trial studies of patients with advanced solid malignancies [64].

Sorafenib (Nexavar®), developed by Bayer, is a drug recently approved for the treatment of advanced RCC. It is a small, oral multikinase inhibitor that targets Raf kinase and several RTKs that induce cell proliferation and/or angiogenesis.. In addition, Sorafenib can inhibit the PDGF and VEGF receptor kinases [65]. In Phase II/III trials sorafenib significantly prolonged progression-free survival of patients with metastatic RCC [66,67].

Ser/Thr kinases targeting drugs

Ser–Thr kinases are involved in various signal transduction pathways transmitting signals of upstream PTKs and playing an essential role in cell proliferation and apoptotic procedures. Although TKs represent the main target for anticancer therapy, a third of the kinase inhibitors developed so far are targeted against Ser/Thr kinases, supporting the idea of being prospective candidates for cancer treatment (**Table 2**).

MK-0457 (VX-680) is a small-molecule inhibitor of Aurora, FLT-3, JAK-2 and BCR-ABL kinases developed by Merck & Co, Inc., and Vertex Pharmaceuticals Inc.. Preclinical

Table 2. Additional kinase-targeting drugs.

Compound	Kinase target(s)	Cancer target(s)
Ser/Thr kinase inhibitors		
MK-0457 (VX-680)	Aurora, FLT-3, JAK-2, BCR-ABL	AML, pancreatic and colon cancer
Affinitak™ (LY900003)	PKC-α	NSCLC
Seliciclib *R*-roscovitine	CDK2, CDK7, CDK9	B-cell lymphomas, NSCLC
ARRY-142886 (AZD6244)	MEK 1/2	Melanoma, pancreatic, colon, lung and breast cancers
Flavopiridol	CDKs	Colorectal cancer, advanced soft-tissue sarcoma
PI3K/Akt pathway inhibitors		
Wortmannin	Akt	NSCLC
LY294002	Akt	Ovarian carcinoma
Archexin (RX-0201)	Akt	Glioblastoma, RCC, ovarian, stomach and pancreatic cancer
Everolimus (Rad001)	mTOR	B-cell lymphomas, breast cancer, RCC
Temsirolimus (CCI-779)	mTOR	Breast cancer, RCC

AML: Acute myelogenous leukemia; mTOR: Mammalian target of rapamycin; NSCLC: Non-small-cell lung cancer; PI3K: Phosphoinositide 3-kinase; RCC: Renal cell carcinoma.

results demonstrated that MK-0457 can induce tumor regression in human models of solid tumor cancers (AML, pancreatic and colon) [68]. Phase I clinical studies for MK-0457 have already initiated in patients with CML [69] to evaluate the safety of MK-0457, to determine the maximum tolerated dose and dose-limiting toxicities, and to assess pharmacokinetics and pharmacodynamics.

LY900003 (Affinitak™), is a specific PKC-α antisense inhibitor developed by Isis Pharmaceuticals. Phase I/II studies showed that Affinitak® has promising survival rates and moderate activity in patients with advanced NSCLC when administered with carboplatin and paclitaxel [70].

R-roscovitine (Seliciclib® or CYC202) is a 2,6,9-substituted purine analogue that inhibits multiple cyclin-dependent kinases including CDK2, CDK7 and CDK9. Developed by Cyclacel, Seliciclib is currently a Phase II clinical trial drug that is used in combination with gemcitabine/cisplatin for the treatment of NSCLC [71], as well as a single agent for B-cell lymphomas.

ARRY-142886 (AZD6244) is a novel, orally available, selective, ATP-noncompetitive inhibitor of MEK 1/2 kinases developed by Array BioPharma Inc.. AZD6244 has shown tumor suppressive activity in multiple preclinical models of human cancer, including melanoma, pancreatic, colon, lung and breast cancers [72,73]. Initiation of a Phase II study for ARRY-142886 for the treatment of stage III/IV malignant melanoma and a variety of other solid tumors has already been announced.

Flavopiridol, a cyclin-dependent kinase inhibitor, has been developed by Aventis Oncology in collaboration with the NCI. Its use is presently under investigation for a variety of solid tumors, as well as hematological cancers. Although flavopiridol has been reported to induce apoptosis in a variety of tumor cells [74], Phase I and II clinical results did not demonstrate flavopiridol to have single-agent activity in patients with various types of cancer [75,76]. However, ongoing studies are examining flavopiridol in combination with other cancer-therapy treatments.

Additional kinase-targeting drugs & novel strategies

PI3K/Akt pathway inhibitors

Phosphoinositide 3-kinases (PI3Ks; lipid kinases) belong to an evolutionarily conserved family of signal transducing enzymes. Activation of PI3K's by growth factors and regulators results in the phosphorylation of phosphoinositides, leading to the transient accumulation of phospholipids in cell membranes [77]. These lipid products serve as second messengers and/or signaling molecules to control many cellular events, such as mitogenic responses, cell differentiation and survival [78,79]. Since abnormalities in the PI3K pathway are common in cancer and have a role in neoplastic transformation [80], it has become an attractive target for the development of novel anticancer agents (**Table 2**). Wortmannin and LY294002 represent two PI3K inhibitors with antitumor activity *in vitro* and *in vivo* [81,82]. Preclinical studies of SF1126 (a broad-spectrum PI3K inhibitor developed by Semafore) has demonstrated good tolerability and promising anticancer activity resulting in the recent initiation of Phase I human clinical trials.

Archexin (RX-0201), is a first-in-class signal inhibitor developed by Rexahn Pharmaceuticals that directly blocks the production of Akt kinase, thereby blocking the proliferation of tumor cells and inducing cell death (apoptosis). In both preclinical and Phase I clinical trials, Archexin has demonstrated effectiveness at inhibiting the proliferation of various cancer cells [83,84].

Rad001 (Everolimus®), an orally available ester derivative of rapamycin developed by Novartis Pharmaceuticals, is a novel downstream multisignal inhibitor that specifically blocks the mTOR protein, a downstream mediator of the PI3K/Akt pathway. In preclinical studies, Everolimus inhibited proliferation and growth of a broad range of human tumor cell lines and xenograft models [85,86]. It is currently being investigated in Phase I and II trials in multiple tumor types and is being developed as an antitumor agent alone [87–89] or in combination with other drugs [90].

CCI-779 (Temsirolimus®; cell-cycle inhibitor-779), another novel inhibitor of mTOR developed by Wyeth Pharmaceuticals, has showed activity in Phase II clinical studies in patients with RCC and glioblastoma and is being evaluated in combination with letrozole in metastatic breast cancer in a Phase III study [91–93].

Farnesylation inhibitors

Farnesylation represents a post-translational modification of proteins that plays an important role in the growth and differentiation of eukaryotic cells. Farnesylation is catalyzed by protein farnesyltransferase (FTase), an enzyme involved in the post-translational modification and activation of Ras proteins. Recent development of farnesyltransferase inhibitors (FTIs) has led to

further insight into the biological significance of farnesylation in cancer cells [94].

Lonafarnib (SCH66336) is a tricyclic nonpeptidomimetic compound that binds to and inhibits FTase. It was developed as an anticancer agent to antagonize oncogenic Ras. Both preclinical and clinical studies have so far shown great antitumor activity. Combinational therapy of lonafarnib with the proteasome inhibitor bortezomib induced synergistic tumor-cell death in multiple myeloma cell lines and primary multiple myeloma plasma cells [95]. Phase I trial studies of combined paclitaxel and lonafarnib treatment demonstrated encouraging clinical activity in solid tumors [96]. Currently, Phase II trials for the therapy of stage III and IV NSCLC are continuing in order to examine whether lonafarnib can increase taxane sensitivity and overcome clinical taxane resistance in solid tumors.

R115777, a new FTI, has demonstrated reduced tumor growth *in vivo* and reduced cell growth *in vitro* in human cancer cell lines, while in human ductal carcinoma *in situ* (DCIS) xenografts treatment with R115777 resulted in a reduced cell turnover index [97]. In addition, a recent Phase I clinical trial of R115777 in combination with gemcitabin and cisplatin in patients with advanced solid tumors has showed evidence of antitumor activity [98].

Heat-shock-protein inhibitors

Heat shock proteins (HSPs), a group of proteins present in all cells, are induced when a cell undergoes various types of environmental stresses. However, they are also present in cells under perfectly normal conditions. HSPs are mainly involved in the proper folding of other proteins and, hence, are referred to as molecular chaperons [99,100]. Most kinases require molecular chaperons to maintain their activation-competent conformation. HSPs interact and stabilize various kinases [101]. Novel strategies are based on the design of inhibitors against HSPs that prevent them from maintaining the activation-competent conformation of the kinase, resulting in the degradation of the misfolded proteins [102].

Outlook & conclusion

It has been proposed that future cancer-therapy strategies must be based on the integration of conventional therapies with novel inhibitors of signals involved in cell proliferation and apoptosis. The new discoveries will help transform oncology from its current state of empirically based patient management to one in which treatment decisions are based on approaches that successfully integrate molecular biology, pathology, imaging and clinical medicine.

Since protein kinases, especially PTKs, play a role in every aspect of cellular function, it is not surprising that more and more of these signaling molecules have become targets for anticancer-drug development in recent years.

Preclinical and clinical data of the 'first generation' TK inhibitors showed remarkable and promising results for the treatment of certain types of cancer where conventional therapeutic methods proved inadequate. The discovery of imatinib/Gleevec against CML showed high remission rates and minimal side effects in almost all treated patients [103]. However, mutation of a single amino acid can make a kinase drug resistant, and mutations in ABL that make it resistant to Gleevec are the cause of relapse in patients who have the advanced stage of CML [104]. Such resistances have presented new therapeutic challenges, leading to the rapid development of the 'second-generation' compounds that can form covalent, permanent bonds with their target and, therefore, increase their effectiveness [105–107]. In addition, certain novel inhibitors possess a multitargeting kinase affinity, allowing them to block multiple signaling pathways with either a combination of agents or a single multitargeted drug.

The future of protein kinase-targeted therapeutics in cancer is promising, despite the fact that several protein-kinase inhibitors that have entered human clinical trials are not very specific and do not express the anticipated results. However, it should be mentioned that protein-kinase inhibitors are important not just for the treatment of disease, but also as reagents to help us understand more about the physiological roles of protein kinases. Many specific protein kinase inhibitors that cannot be used as drugs for reasons of toxicity [108,109], pharmacology or solubility could be extremely useful research reagents.

Although cancer is clearly a polygenic disease, the effectiveness of certain kinase inhibitors against hematologic and solid tumors reported so far suggests that certain tumors are remarkably dependent on activation of a single kinase. Therefore, in the following years, research will focus even more on the development of kinase inhibitors and other 'molecular-targeted' therapies as well as in combinational therapies, in order to counteract the problem of resistance, resulting in greater antitumor activity.

However, in our approach for the development of these drugs, we need to conscientiously incorporate assays to assess the suitability of the patient population, the target and the effects of the target in order to improve the efficiency of the evaluation of an agent and the probability of success. In that way, in the coming future, such inhibitors, covering most of the kinases involved in the various cancers, will make a significant difference in disease outcome.

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Executive summary

- Protein phosphorylation is a post-translational modification, regulated by kinases, that plays an essential role in the control and regulation of fundamental cellular processes (i.e., the cell cycle, proliferation, and cell death or survival). It has been shown that the abnormal phosphorylation of proteins can lead to the development of a number of disorders and major diseases, including cancer.
- Development of drugs against protein kinases represents a novel and promising approach to cancer therapy. More precisely, tyrosine kinases (TKs) have emerged as clinically useful drug-target molecules for treating certain types of cancer, since unregulated activation of these enzymes can lead to various forms of cancer.
- So far, different approaches used to target kinases have resulted in the production of various compounds including: humanized monoclonal antibodies, inhibitors of angiogenesis, small molecule inhibitors, farnesylation inhibitors and heat-shock-protein inhibitors.
- Imatinib mesylate (Gleevec®) represents the first highly successful TK inhibitor that was used as a cancer drug in patients with chronic myelogenous leukemia. However, despite the early successes, the majority of responding patients have developed secondary resistance to Gleevec.
- Kinases targeted by novel inhibitory molecules include EGF receptors, VEGF receptors, ABL1/2, PDGF receptors, KIT, Src family, FLT3, RAF1, BRAF, Aurora, JAK-2, MEK ½, CDKs, Akt and mTOR.
- As kinases can mediate many signaling pathways, the design of drugs targeting a unique kinase with precise specificity seems unfeasible. Therefore, pharmaceutical companies are focusing on the development of drugs with multiple effects to overcome the fact that tumors have many biological paths they can use to grow, resist death and spread.
- Integration of conventional therapies (surgery, radiation and chemotherapy) with novel kinase inhibitors and other 'molecular targeted' therapies could prove to be a successful strategy for treating cancer in the future.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. *CA Cancer J. Clin.* 55(2), 74–108 (2005).
- **Estimates of the worldwide incidence, mortality and prevalence of 26 cancers in the year 2002.**
2. Fabbro D, Ruetz S, Buchdunger E *et al.*: Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. *Pharmacol. Ther.* 93(2–3), 79–98 (2002).
3. Levitzki A: Protein kinase inhibitors as a therapeutic modality. *Acc. Chem. Res.* 36(6), 462–469 (2003).
4. Pratt DJ, Endicott JA, Noble ME: The role of structure in kinase-targeted inhibitor design. *Curr. Opin. Drug Discov. Devel.* 7(4), 428–436 (2004).
5. Cohen P: Protein kinases – the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* 1(4), 309–315 (2002).
- **Review of some of the most important advances in the development and use of protein kinases as drug targets.**
6. Vieth M, Higgs RE, Robertson DH, Shapiro M, Gragg EA, Hemmerle H: Kinomics – structural biology and chemogenomics of kinase inhibitors and targets. *Biochim. Biophys. Acta* 1697(1–2), 243–257 (2004).
7. Kennelly PJ: Archaeal protein kinases and protein phosphatases: insights from genomics and biochemistry. *Biochem. J.* 370(Pt 2), 373–389 (2003).
8. Kostich M, English J, Madison V *et al.*: Human members of the eukaryotic protein kinase family. *Genome Biol.* 3(9), RESEARCH0043 (2002).
9. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S: The protein kinase complement of the human genome. *Science* 298(5600), 1912–1934 (2002).
- •• **Catalog of the protein kinase complement of the human genome, including information concerning the classification of protein kinases, comparison with model organism kinomes and chromosomal mapping of kinase genes.**
10. Cohen P: The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. *Eur. J. Biochem.* 268(19), 5001–5010 (2001).
- **Overview of the progress that is being made in developing specific inhibitors of protein kinases for the treatment of cancer and chronic infammatory diseases.**
11. Blume-Jensen P, Hunter T: Oncogenic kinase signaling. *Nature* 411, 355–365 (2001).
12. Hunter T: Signaling – 2000 and beyond. *Cell* 100(1), 113–127 (2000).

13. Ventura JJ, Nebreda AR: Protein kinases and phosphatases as therapeutic targets in cancer. *Clin. Transl. Oncol.* 8(3), 153–160 (2006).
14. Ross JS, Fletcher JA, Linette GP *et al.*: The Her-2/neu gene and protein in breast cancer 2003: biomarker and target of therapy. *Oncologist* 8(4), 307–325 (2003).
15. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J: Structural mechanism of STI-571 inhibition of Abelson tyrosine kinase. *Science* 289(5486), 1938–1942 (2000).

•• **Report providing information regarding the crystal structure of the catalytic domain of Abl complexed to STI-571, explaining the inhibitory functioning mechanism.**

16. Demetri GD, von Mehren M, Blanke CD *et al.*: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 347(7), 472–480 (2002).
17. Buchdunger E, Cioffi CL, Law N *et al.*: Abl protein-tyrosine kinase inhibitor, STI-571, inhibits *in vitro* signal transduction mediated by c-kit and PDGF receptors. *J. Pharmacol. Exp. Ther.* 295(1), 139–145 (2000).
18. Buchdunger E, Zimmermann J, Mett H *et al.*: Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. *Proc. Natl Acad. Sci. USA* 92(7), 2558–2562 (1995).
19. Druker BJ, Tamura S, Buchdunger E *et al.*: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med.* 2(5), 561–566 (1996).
20. Fabian MA, Biggs WH 3rd, Treiber DK *et al.*: A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* 23(3), 329–336 (2005).
21. Weiss A, Schlessinger J: Switching signals on or off by receptor dimerization. *Cell* 94(3), 277–280 (1998)
22. Xu W, Harrison SC, Eck MJ: Three-dimensional structure of the tyrosine kinase c-Src. *Nature* 385, 595–602 (1997).
23. Thomas SM, Brugge JS: Cellular functions regulated by Src family kinases. *Annu. Rev. Cell Dev. Biol.* 13, 513–609 (1997).
24. Schlessinger J: Cell signaling by receptor tyrosine kinases. *Cell* 103(2), 211–225 (2000).

• **Review explaining mechanisms of activation of signaling pathways by receptor tyrosine kinases.**

25. Zwick E, Bange J, Ullrich A: Receptor tyrosine kinase as targets for anticancer drugs. *Trends Mol. Med.* 8(1), 17–23 (2002).
26. Smith KM, Yakobi R, Van Etten RA: Autoinhibition of Bcr-Abl through its SH3 domain. *Mol. Cell* 12(1), 27–37 (2003).
27. Tateishi M, Ishida T, Mitsudomi T, Kaneko S, Sugimachi K: Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. *Cancer Res.* 50(21), 7077–7080 (1990).
28. Watanabe D, Ezoe S, Fujimoto M *et al.*: Suppressor of cytokine signaling-1 gene silencing in acute myeloid leukemia and human haematopoietic cell lines. *Br. J. Haematol.* 126(5), 726–735 (2004).
29. Riethmuller G, Schneider-Gadicke E: Johnson JP: Monoclonal antibodies in cancer therapy. *Curr. Opin. Immunol.* 5(5), 732–739 (1993).
30. Bennasroune A, Gardin A, Aunis D, Cremel G, Hubert P: Tyrosine kinase receptors as attractive targets for cancer therapy. *Crit. Rev. Oncol. Hematol.* 50(1), 23–28 (2004).
31. Houshmand P, Zlotnik A: Targeting tumor cells. *Curr. Opin. Cell Biol.* 15(5), 640–644 (2003).
32. Slamon DJ, Leyland-Jones B, Shak S *et al.*: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 344(11), 783–792 (2001).

• **Demonstrates that trastuzumab can increase the clinical benefit of first-line chemotherapy in metastatic breast cancer that overexpresses HER2.**

33. Cunningham D, Humblet Y, Siena S *et al.*: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351(4), 337–345 (2004).
34. Hoy SM, Wagstaff AJ: Panitumumab: in the treatment of metastatic colorectal cancer. *Drugs* 66(15), 2005–2014 (2006).
35. Berlin J, Posey J, Tchekmedyian S *et al.*: Panitumumab with irinotecan/leucovorin/5-fluorouracil for first-line treatment of metastatic colorectal cancer. *Clin. Colorectal Cancer* 6(6), 427–432 (2007).
36. Sridhar SS, Shepherd FA: Targeting angiogenesis: a review of angiogenesis inhibitors in the treatment of lung cancer. *Lung Cancer* 42 (Suppl. 1), S81–S91 (2003).
37. Levitzki A: Tyrosine kinases as targets for cancer therapy. *Eur. J. Cancer* 38(Suppl. 5), S11–S18 (2002).
38. Levitzki A: Tyrphostins – potential antiproliferative agents and novel molecular tools. *Biochem. Pharm.* 40(5), 913–918 (1990).
39. Daub H, Specht K, Ullrich A: Strategies to overcome resistance to targeted protein kinase inhibitors. *Nat. Rev. Drug Discov.* 3(12), 1001–1010 (2004).
40. Rubin B, Duensing A: Mechanisms of resistance to small molecule kinase inhibition in the treatment of solid tumors. *Lab. Invest.* 86(10), 981–986 (2006).
41. Pao W, Miller VA, Politi KA *et al.*: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* 2(3), e73 (2005).
42. Cohen MH, Williams GA, Sridhara R *et al.*: United states food and drug administration drug approval summary: gefitinib (ZD1839; Iressa) tablets. *Clin. Cancer Res.* 10, 1212–1218 (2004).
43. Kobayashi S, Boggon TJ, Dayaram T *et al.*: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* 352(8), 786–792 (2005).
44. Kwak EL, Sordella R, Bell DW *et al.*: Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Natl Acad. Sci. USA* 102(21), 7665–7670 (2005).
45. Fletcher JA, Corless CL, Dimitrijevic S *et al.*: Mechanisms of resistance to imatinib mesylate (IM) in advanced gastrointestinal stromail tumor (GIST). *Proc. Am. Soc. Clin. Oncol.* 22, 3275 (2003).
46. Debiec-Rychter M, Cools J, Dumez H *et al.*: Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. *Gastroenterology* 128(2), 270–279 (2005).
47. Burris HA 3rd, Hurwitz HI, Dees EC *et al.*: Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. *J. Clin. Oncol.* 23(23), 5305–5313 (2005).
48. Konecny GE, Pegram MD, Venkatesan N *et al.*: Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res.* 66(3), 1630–1639 (2006).
49. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL: Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 305(5682), 399–401 (2004).

50. Guilhot F, Apperley J, Kim DW *et al.*: Dasatinib induces significant hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in accelerated phase. *Blood* (2007) (In press).
51. Golemovic M, Verstovsek S, Giles F *et al.*: AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has *in vitro* activity against imatinib-resistant chronic myeloid leukemia. *Clin.Cancer Res.* 11(13), 4941–4947 (2005).
52. O'Hare T, Walters DK, Stoffregen EP *et al.*: *In vitro* activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res.* 65(11), 4500–4505 (2005).
53. Weisberg E, Manley PW, Breitenstein W *et al.*: Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* 7(2), 129–141 (2005).
54. Kantarjian H, Giles F, Wunderle L *et al.*: Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N. Engl. J. Med.* 354(24), 2594–2596 (2006).
- **Demonstrates that the tyrosine kinase inhibitor nilotinib has a relatively favorable safety profile and was active in imatinib-resistant chronic myelogenous leukemia.**
55. Mulder PH, Roigas J, Gillessen S, *et al.*: A phase II study of sunitinib administered in a continuous daily regimen in patients with cytokine-refractory metastatic renal cell carcinoma (mRCC) [Abstract]. *J. Clin. Oncol.* 24, 4529 (2006).
56. Motzer RJ, Michaelson MD, Redman BG *et al.*: Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 24(1), 16–24 (2006).
57. Motzer RJ, Hutson TE, Tomczak P *et al.*: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N. Engl. J. Med.* 356(2), 115–124 (2007).
58. Soria JC, Zurlo A, Blay JY *et al.*: Final results of a Phase I trial of Masitinib (AB1010) in solid tumors. *AACR Annual meeting* (2007).
59. (Bui BN, Blay J, Duffaud F *et al.*: Preliminary efficacy and safety results of Masitinib administered, front line in patients with advanced GIST. A phase II study. *J. Clin. Oncol. ASCO Annu. Meeting Proc. Part I.* 25, S18 (June 20 Supplement) (2007).
60. Stavropoulos JA, Meulbroek JA, Niquette AL *et al.*: Preclinical activity of ABT-869, a novel receptor tyrosine kinase inhibitor, alone or in combination with paclitaxel. *J. Clin. Oncol. 2005 ASCO Annu. Meeting Proc.* 23, S16 (June 1 Supplement) (2005).
61. Shankar DB, Li J, Tapang P *et al.*: ABT-869, a multitargeted receptor tyrosine kinase inhibitor: inhibition of FLT3 phosphorylation and signaling in acute myeloid leukemia. *Blood* 109(8), 3400–3408 (2007).
62. Knapper S, Mills KI, Gilkes AF *et al.*: The effects of lestaurtinib (CEP701) and PKC412 on primary AML blasts: the induction of cytotoxicity varies with dependence on FLT3 signaling in both FLT3-mutated and wild-type cases. *Blood* 108(10), 3494–3503 (2006).
63. Knapper S, Burnett AK, Littlewood T *et al.*: A Phase 2 trial of the FLT3 inhibitor lestaurtinib (CEP701) as first-line treatment for older patients with acute myeloid leukemia not considered fit for intensive chemotherapy. *Blood* 108(10), 3262–3270 (2006).
64. Wakelee H, Adjei AA, Keer H *et al.*: A Phase I dose-escalation and pharmacokinetic (PK) study of a novel multiple-targeted receptor tyrosine kinase (RTK) inhibitor, XL647, in patients with advanced solid malignancies. *J. Clin. Oncol. 2005 ASCO Annu. Meeting Proc. Part I* 23, S16 (June 1 Supplement) (2005),
65. Wilhelm SM, Carter C, Tang L *et al.*: BAY 43–9006 exhibits broad spectrum oral anti-tumor activity and targets the Raf/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res.* 64(19), 7099–7109 (2004).
66. Ratain MJ, Eisen T, Stadler WM *et al.*: Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 24(16), 2505–2512 (2006).
67. Escudier B, Eisen T, Stadler WM *et al.*: Sorafenib in advanced clear-cell renal-cell carcinoma. *N. Engl. J. Med.* 356(2), 125–134 (2007).
- **Patients with advanced clear-cell renal-cell carcinoma, where previous therapy has failed, were treated with sorafenib and demonstrated a prolonged progression-free survival.**
68. Harrington EA, Bebbington D, Moore J *et al.*: Addendum: VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth *in vivo*. *Nat. Med.* 13(4), 511 (2007).
69. Giles FJ, Cortes J, Jones D, Bergstrom D, Kantarjian H, Freedman SJ: MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. *Blood* 109(2), 500–502 (2007).
70. Villalona-Calero MA, Ritch P, Figueroa JA *et al.*: A Phase I/II study of LY900003, an antisense inhibitor of protein kinase C-α, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer. *Clin. Cancer Res.* 10(18 Pt 1), 6086–6093 (2004).
71. Siegel-Lakhai WS, Rodenstein DO, Beijnen JH *et al.*: Phase I study of seliciclib (CYC202 or R-roscovitine) in combination with gemcitabine (gem)/cisplatin (cis) in patients with advanced non-small cell lung cancer (NSCLC). *J. Clin. Oncol. ASCO Annu. Meeting Proc. Part I* 23, S16 (June 1 Supplement) (2005).
72. Sebolt-Leopold JS, Dudley DT, Herrera R *et al.*: Blockade of the MAP kinase pathway suppresses growth of colon tumors *in vivo*. *Nat. Med.* 5(7), 810–816 (1999).
73. Winkler J, Lee P, Wallace E *et al.*: Anti-tumor activity, pharmacokinetic and pharmacodynamic effects of the MEK inhibitor ARRY-142886 (AZD6244) in a BxPC3 pancreatic tumor xenograft model [abstract]. *Proc. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics*, Geneva, Switzerland (2004).
74. Sedlacek HH: Mechanisms of action of flavopiridol. *Crit. Rev. Oncol. Hematol.* 38(2), 139–170 (2001).
75. Aklilu M, Kindler H L, Donehower RC, Mani S, Vokes EE: Phase II study of flavopiridol in patients with advanced colorectal cancer. *Annals of Oncology.* 14, 1270–1273 (2003).
76. Morris DG, Bramwell VH, Turcotte R *et al.*: A Phase II study of flavopiridol in patients with previously untreated advanced soft tissue sarcoma. *Sarcoma*, 64374 (2006) (Epub ahead of print).
77. Fruman DA, Meyers RE, Cantley LC: Phosphoinositide kinases. *Annu. Rev. Biochem.* 67, 481–507 (1998).
78. Martin TF: Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation, and membrane trafficking. *Annu. Rev. Cell Dev. Biol.* 14, 231–264 (1998).
79. Okkenhaug K, Vanhaesebroeck B: PI3K in lymphocyte development, differentiation and activation. *Nat. Rev. Immunol.* 3, 317–330 (2003).
80. Hanahan D, Weinberg RA: The hallmarks of cancer. *Cell* 100, 57–70 (2000).

81. Hu L, Zaloudek C, Mills, GB, Gray J, Jaffe RB: *In vivo* and *in vitro* ovarian carcinoma growth inhibition by a phosphatidylinositol 3-kinase inhibitor (LY294002). *Clin. Cancer Res.* 6, 880–886 (2000).
82. Ng SS, Tsao, MS, Nicklee T, Hedley DW: Wortmannin inhibits pkb/akt phosphorylation and promotes gemcitabine antitumor activity in orthotopic human pancreatic cancer xenografts in immunodeficient mice. *Clin. Cancer Res.* 7, 3269–3275 (2001).
83. Marshall JJ, Posey J, Hwang J *et al.*: A Phase I trial of RX-0201 (AKT anti-sense) in patients with an advanced cancer. *J. Clin. Oncol. 2007 ASCO Annu. Meeting Proc.* 25, S18 (June 20 Supplement) (2007).
84. Malik SM, Hwang J, Marshall J, *et al.*: Phase I study of RX-0201 in patients with advanced or metastatic solid tumors. *J. Clin. Oncol. ASCO Annu. Meeting Proc.* 24, S18 (June 20 Supplement) (2006).
85. Boulay A, Zumstein-Mecker S, Stephan C *et al.*: Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. *Cancer Res.* 64(1), 252–261 (2004).
86. Aguirre D, Boya P, Bellet D *et al.*: Bcl-2 and CCND1/CDK4 expression levels predict the cellular effects of mTOR inhibitors in human ovarian carcinoma. *Apoptosis* 9(6), 797–805 (2004).
87. Lerut E, Roskams T, Goossens E *et al.*: Molecular pharmacodynamic evaluation of dose and schedule of RAD001 (everolimus) in patients with operable prostate carcinoma [abstract 3071]. *Proc. Am. Soc. Clin. Oncol. Annu. Meet.* 23, S209 (2005).
88. Tabernero J, Rojo F, Burris H *et al.*: A Phase I study with tumor molecular pharmacodynamic evaluation of dose and schedule of the oral mTOR-inhibitor Everolimus (RAD001) in patients with advanced solid tumors [abstract 3007]. *Proc. Am. Soc. Clin. Oncol. Annu. Meet.* 23, S193 (2005).
89. O'Donnell A, Faivre S, Judson I *et al.*: A Phase I study of the oral mTOR inhibitor RAD001 as monotherapy to identify the optimal biologically effective dose using toxicity, pharmacokinetic, and pharmacodynamic endpoints in patients with solid tumors [abstract 803]. *Proc. Am. Soc. Clin. Oncol. Annu. Meet.* 22, 200 (2003).
90. Yao JC, Phan AT, Chang DZ et al.: Phase II study of RAD001 (everolimus) and depot octreotide (Sandostatin LAR) in patients with advanced low grade neuroendocrine carcinoma (LGNET). *J. Clin. Oncol. ASCO Annu. Meeting Proc. Part I.* 24, S18 (June 20 Supplement) (2006).
91. Atkins MB, Hidalgo M, Stadler WM *et al.*: Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J. Clin. Oncol.* 22, 909–918 (2004).
92. Chan S, Johnston S, Scheulen ME *et al.*: First report: a phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. *Proc. Am. Soc. Clin. Oncol.* 21, 175 (2002).
93. Galanis, E, Buckner JC, Maurer M *et al.*: NCCTG phase II trial of CCI-779 in recurrent glioblastoma multiforme (GBM). *Proc. Am. Soc. Clin. Oncol.* 22, 1503 (2004).
94. Tamanoi F, Gau CL, Jiang C, Edamatsu H, Kato-Stankiewicz J: Protein farnesylation in mammalian cells: effects of farnesyltransferase inhibitors on cancer cells. *Cell Mol. Life Sci.* 58(11), 1636–1649 (2001).
95. David E, Sun SY, Waller EK, Chen J, Khuri FR, Lonial S: The combination of the farnesyl transferase inhibitor lonafarnib and the proteasome inhibitor bortezomib induces synergistic apoptosis in human myeloma cells that is associated with down-regulation of p-AKT. *Blood* 106(13), 4322–4329 (2005).
96. Khuri FR, Glisson BS, Kim ES *et al.*: Phase I study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in solid tumors. *Clin. Cancer Res.* 10(9), 2968–2976 (2004).
97. Wärnberg F, White D, Anderson E, Knox F, Clarke RB, Morris J, Bundred NJ: Effect of a farnesyl transferase inhibitor (R115777) on ductal carcinoma *in situ* of the breast in a human xenograft model and on breast and ovarian cancer cell growth *in vitro* and *in vivo*. *Breast Cancer Res.* 8(2), R21 (2006).
98. Adjei AA, Croghan GA, Erlichman C *et al.*: A Phase I trial of the farnesyl protein transferase inhibitor R115777 in combination with gemcitabine and cisplatin in patients with advanced cancer. *Clin. Cancer Res.* 9(7), 2520–2526 (2003).
99. Bukau B, Horwich AL: The Hsp70 and Hsp60 chaperon machines. *Cell* 92(3), 351–356 (1998).
100. Hartl FU: Molecular chaperones in cellular protein folding. *Nature.* 381(6583), 571–579 (1996).
101. Yarden, Y, Sliwkowski MX: Untangling the ErbB signaling network. *Nature Rev. Mol. Cell Biol.* 2(2), 127–137 (2001).
102. Demetri GD, George S, Van Den Abbeele A *et al.*: Inhibition of heat shock protein 90 (Hsp90) with the novel agent IPI-504 to overcome resistance to tyrosine kinase inhibitors (TKIs) in metastatic GIST: Results of a phase I trial. *Gastrointestinal Cancers Symposium* (2007).
103. Druker BJ, Talpaz M, Debra J *et al.*: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* 344, 1031–1037 (2001).
104. Gorre ME, Mohammed M, Ellwood K *et al.*: Clinical resistance to STI-571 cancer therapy caused by *BCR-ABL* gene mutation or amplification. *Science* 293(5531), 876–880 (2001).
105. Wissner A, Overbeek E, Reich MF *et al.*: Synthesis and structure-activity relationships of 6,7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design of an orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2). *J. Med. Chem.* 46, 49–63 (2003.).
106. Rabindran SK, Discafani CM, Rosfjord EC *et al.*: Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.* 64, 3958–3965 (2004).
107. Allen LF, Eiseman IA, Fry DW *et al.*: CI-1033, an irreversible pan-ErbB receptor inhibitor and its potential application for the treatment of breast cancer. *Semin. Oncol.* 30, 65–78 (2003).
108. Force T, Krause DS, Van Etten RA: Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nature Rev. Cancer* 7, 332–344 (2007).
109. Robert C, Soria JC, Spatz A *et al.*: Cutaneous side-effects of kinase inhibitors and blocking antibodies. *Lancet Oncol.* 6(7), 491–500 (2005).